Synopsis

Title: Development and Characterization of Naringenin- Loaded Hydrogel Film for Wound healing

INTRODUCTION

Chronic, non-healing diabetic wounds put a massive economic burden on health services causing patient incompliance and discomfort. Thorough interpreting of chronic wound pathophysiology led to the fabrication of targeted systems of drug delivery that can improve and accelerate the wound healing process [1]. Minor injuries to skin may heal quickly, but full thickness wounds are often infected with multiple microbes, inflammation, poor blood supply and oxygenation that may lead to delayed wound healing. As wounds are prone to microbial invasion, so it is imperative to choose wound dressing that provide occlusive barrier against infectious agents, inhibits microbial growth and promotes skin healing [2]. Traditional wound dressings include cotton, wool, bandages, lint, and gauzes. These dry dressings have limited capacity to absorb fluid exudates, permeable to bacteria, insufficient wound coverage, and may cause secondary injuries by adherence to the wound. An ideal wound healing dressing would maintain moist environment to facilitate the healing process with minimal scarring [3]. Hydrogels are swollen three-dimensional (3D) porous cross-linked networks capable of imbibing huge amounts of water or biological fluids. Due to their biocompatible nature, diverse architecture, flexible production methods and appropriate physical features, hydrogels are widely employed and useful material in the biomedical sector [4,5]. Polysaccharides make up the preponderance of biomaterials found in nature. Several natural or synthetic materials and their blends are used to develop hydrogel films such as, Gelatine, alginate methylcellulose, carboxymethylcellulose, acacia gum, sodium alginate, polyvinyl alcohol, chitosan, collagen, pectin, gellan gum, xanthan gum, and carrageenan gum. Natural polymers are often preferred as they are biodegradable and biocompatible [6]. Synthetic polymers, although, have good thermal stability and mechanical strength, but they may be toxic due to presence of residual monomers. These hydrogel films if loaded good antioxidant or anti-inflammatory drug will show more potential for prompt wound healing [7]. Naringenin belongs to flavanone class of flavonoids, and it is mainly found in citrus fruits including, lemon, orange, tangerine and grapefruit. The antioxidant activity is the most recognized effect of flavonoids, which depends, for instance, on hydrogen donation and electron stabilization in the phenolic rings of inflammatory pain. Naringenin inhibits the pain-like behavior induced by inflammatory stimuli such as phenyl-p-benzoquinone, acetic acid, formalin, complete Freund's adjuvant, capsaicin, carrageenan, superoxide anion, and LPS. Moreover, naringenin inhibits UVB irradiation-induced skin inflammatory edema, cytokine production, myeloperoxidase activity, matrix metalloproteinase-9 activity, and oxidative stress [8].

Naringenin inhibits leukocyte recruitment and production of superoxide anion, whilst increases GSH, and antioxidant capacity. Naringenin also acts on macrophages inducing Nrf2 activation, a nuclear factor that induces antioxidant and anti-inflammatory responses, inducing HO-1 expression [9].

Rationale of the study: Sodium alginate and chitosan, natural biopolymers known for their biocompatibility, biodegradability, and hemostatic properties, have shown significant potential

in wound healing applications. Their antibacterial, anti-inflammatory, and extracellular matrix (ECM)-mimicking properties make them ideal candidates for chronic wound care. These polymers aid in rapid hemostasis, absorb exudates, reduce bacterial infection, and promote collagen deposition and neovascularization. In this study, it is hypothesized that Na-alginate and chitosan-based hydrogel membranes will serve as effective wound dressings by forming a protective barrier, supporting clot formation, and maintaining a moist environment conducive to healing. The current work aims to evaluate and compare the efficacy and safety of Na-alginate and Na-alginate/chitosan hydrogels through comprehensive in vitro, in vivo, and stability studies.

OBJECTIVE:

- To provide moisture environment to enhance the healing of chronic wound
- To enhance anti-inflammatory and antioxidant drug action
- To prolong the release of drug
- To eradicate bacterial infection
- To promote tissue regeneration by granulation and re-epithelization

Novelty:

This novel combination of polymers like sodium with naringenin-loaded hydrogel will provide robust healing action as squishy, absorbent alginates are used to develop hydro fibers dressings that produce a soft gel whenever they contact the wound exudates. This polysaccharide has more absorbent capacity which makes it highly effective for intermediate to severely exude wounds. However, due to the low mechanical strength of alginate hydrogels, they are blended with other polymeric materials like PVA or chitosan. So, in this research investigation sodium alginate was blended with PVA to increase mechanical strength and in second formulation it was blended with chitosan to enhance the healing in addition to the healing effect of naringenin as anti-inflammatory agent in the first formulation. The second formulation was designed using chitosan and sodium alginate to provide prompt wound closure. These novel formulations will target MMP-9 and will provide antibacterial properties.

- **Selection and procurement of drug and chemicals**
- Pre-formulation study of drug
  - Organoleptic properties
  - Melting point
  - Fourier-transformed infrared (FT-IR) spectroscopy
  - X-Ray Diffraction (XRD)
  - Differential scanning calorimetry (DSC)
  - Partition coefficient
  - Solubility via UV spectroscopy
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- **Preparation of naringenin containing Hydrogel film**
- **Characterization of Naringenin containing Hydrogel film**
  - Physical characterization
  - Water vapors permeability
  - Swelling ratio
  - Mechanical properties

- Fourier-transformed infrared (FT-IR) spectroscopy
- Entrapment efficiency
- Gel fraction
- SEM

❖ Anti-bacterial growth activity (*S. aureus and E. coli*)
❖ Cell cytotoxicity study(L929)
❖ Histological examination
❖ Wound healing rate in Male Wistar Rats
- Wound Contraction and re-epithelization
- Hydroxyproline content

Expected outcome

Naringenin-loaded hydrogel film will help in prompt healing of chronic wounds as it will provide moist action thereby minimizing pain at the wound site. Biopolymers used in the formulation are biocompatible and mimic the ECM with deposition of collagen fibers so help in rapid healing. This novel formulation will provide sustained action of the drug and combining the drug with the unique combination of these polymers will be beneficial for effective and faster healing.

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